DEVELOPMENTAL MORPHOLOGY OF ASCOMYCETES

VII. *FENNELLIA FLAVIPES*

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This paper is the seventh in a series on the developmental morphology of Ascomycetes and deals with *Fennellia flavipes* Wiley & Simmons. The culture used in the present study is the type and it was received from Dr. E.G. SIMMONS, Department of Botany, University of Massachussetts, Amherst, USA, under the N° QM 9131.

The fungus was grown on malt extract agar medium and the various stages of the teliomorph development were studied by making tease mounts stained with 0.1% lactofuchsin as recommended by CARMICHAEL (1955) and sectioning the material by the paraffin method as described by JOHANSEN (1940) and PURVIS, COLLIER and WALLS (1964). For the study of the anamorph, RIDDELL's (1950) slide culture technique was used and the fungus was stained with 0.5% solution of trypan blue in lactophenol (BOOTH, 1971) for 15 minutes.

DESCRIPTION OF THE FUNGUS

Colonies on malt extract agar at room temperature (23-25°C) attaining a diameter of 6 cm in 20 days, Lemon chrome in colour, consisting of a basal felt with a few radiating sectors. Exudate present.

Hyphae more or less hyaline to pale yellow, branched, variable in thickness, remotely to closely septate. Racquette hyphae present. Hyphal fusions often noticed between ordinary hyphae, or narrow hyphae, or between ordinary

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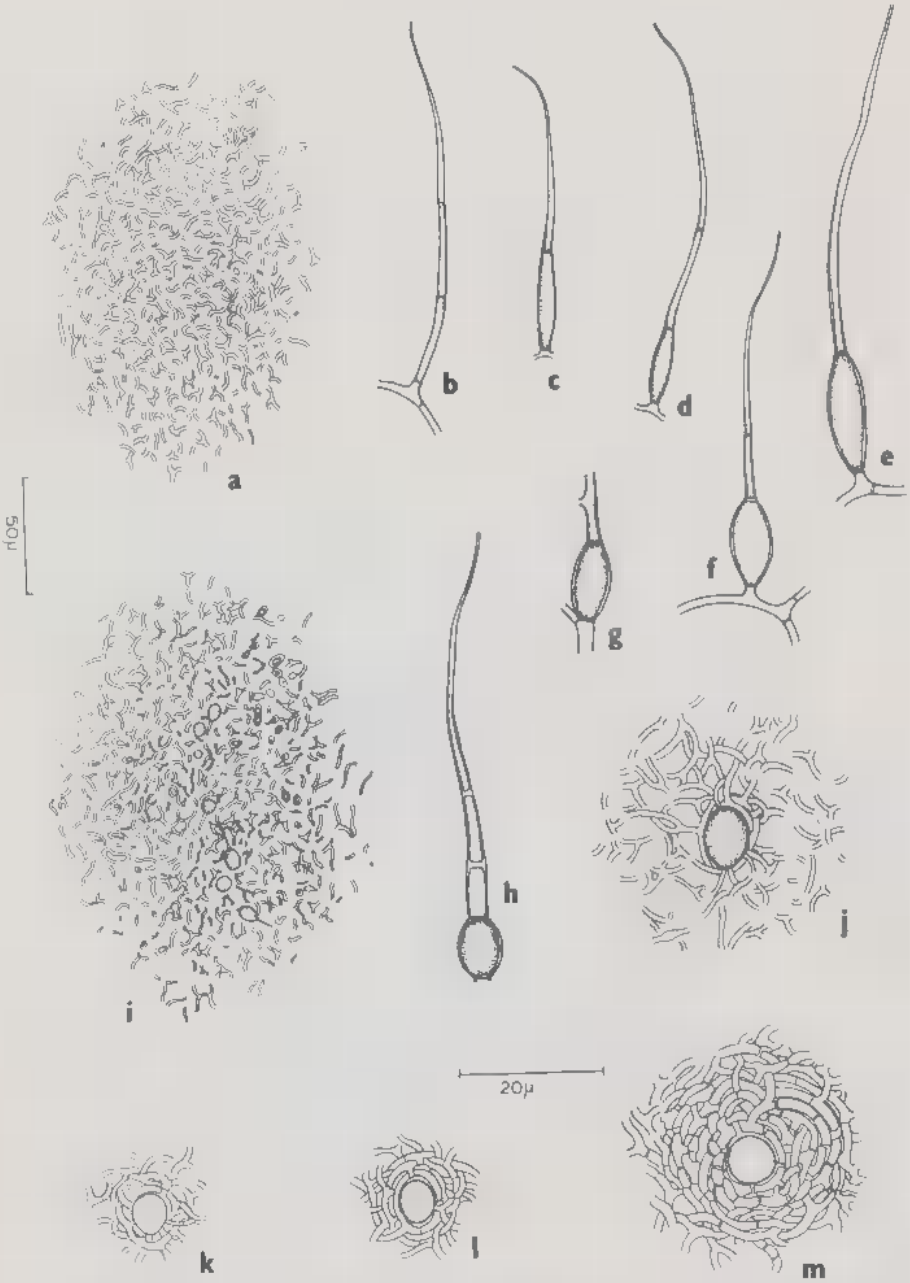


Fig. 1. - *Pennellia flavipes*. a: early stage in the development of a stroma (section); b-h: stages in the development of the ascogonium; i: section of a stroma showing ascogonia and development of Hülle cells in the periphery; j-m:: stages in the development of an ascocarp (sections) - note the ascogonium in the centre.

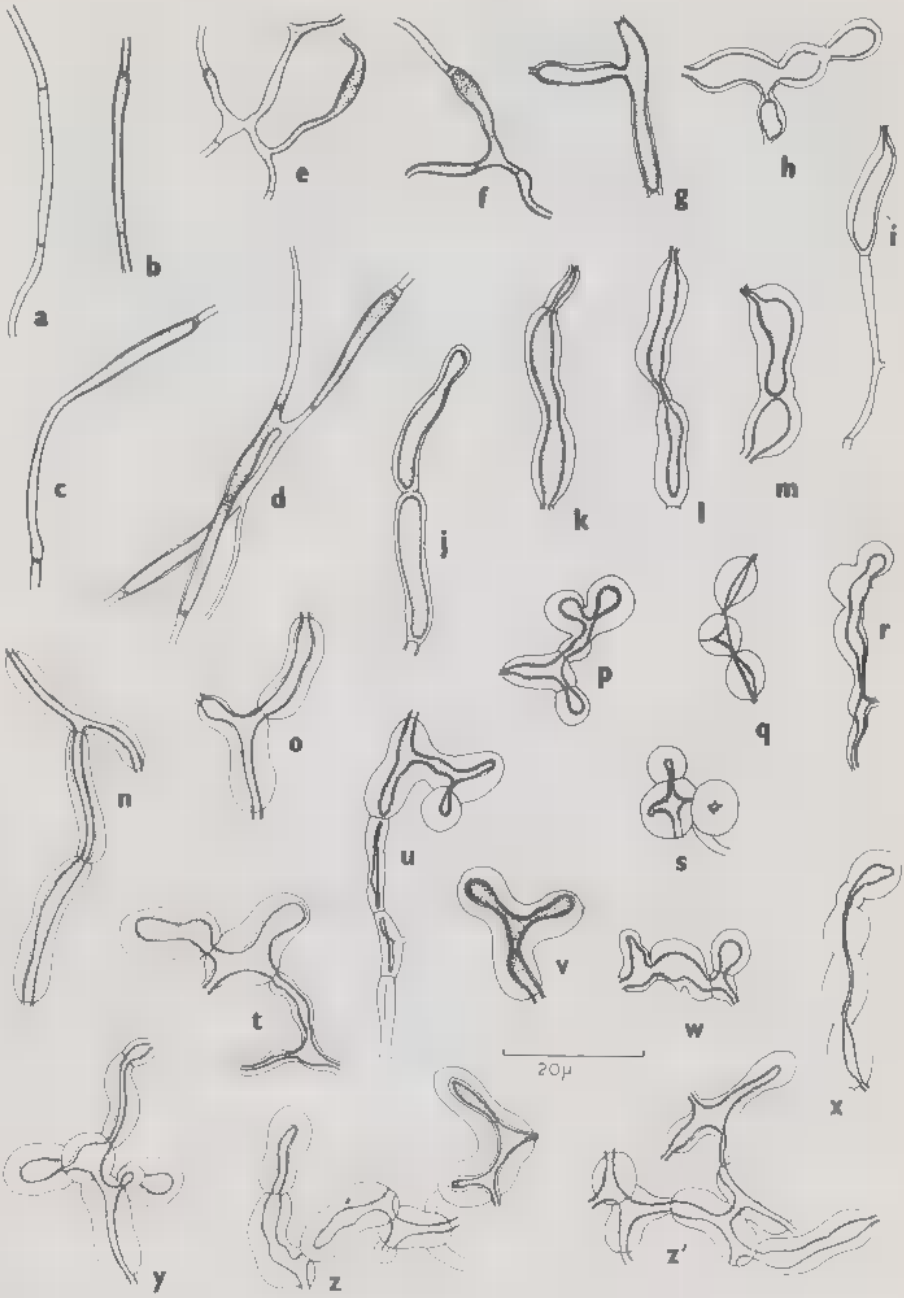


Fig. 2. - *Fennellia flavipes*. a-z': stages in the development of Hülle cells.

hyphae and racquette hyphae. Ascocarps developing within ascostroma. Ascostroma globose to elongate, prosenchymatous, enveloped by masses of Hülle cells, up to 600 x 850μm. Hülle cells rounded to elongate, Pinard yellow to Empire yellow in colour, up to 22.5μm long, 4-8μm broad (Fig. 2, a-z). Ascogonia produced within stroma, 1-15 in number, globose to subglobose or sometimes elongate, each with a short stalk and a straight, septate trichogyne-like extension up to 50μm long; globose ascogonia 7-8μm in diameter; elongate ascogonia up to 20μm long and 5.0-7.5μm wide (Fig. 1, c-h; Plate I, 1). Mature ascocarps 60-160μm long and 50-100μm broad, each with a peridium consisting of a few layers of thin walled narrow cells. Asci formed from croziers, in groups, irregularly disposed, globose to subglobose, thin-walled, mostly with 4 ascospores each, occasionally with eight, 10-12.5μm in diameter (Fig. 5, i-l). Ascospores hyaline to pale yellow, globose to subglobose, smooth, 6-8 x 5-7μm, each with an inconspicuous equatorial groove, tending to stick together in groups (Fig. 6, c & d). Conidial heads radiate to loosely columnar (Fig. 7, v; Plate I, 8), white or nearly so. Phialophores up to 900μm long and normally 5-8μm wide, smooth, subhyaline with an apical vesicle. Vesicles globose to subglobose, and phialides. Metulas mostly 4-6μm long. Phialides produced in groups of a few at the tip of each metula, mostly 5-9 x 2.5-3.0μm. Conidia typically dry, globose, smooth, 2.5-3.5μm in diameter and catenate (Fig. 7, w). Gangliar conidia present, globose, produced singly or in groups from normal hyphae or racquette hyphae.

DEVELOPMENT OF THE TELIOMORPH

The development of the teliomorph is observed in 4-5 days old cultures on malt extract agar medium, and is marked by the production of a prosenchymatous stroma, formed by the aggregation of a group of profusely branched interwoven system of narrow hyphae produced from the vegetative mycelium (Fig.1, a).

A few short, erect, setose hyphal branches develop within the stroma. These branches grow to a length of around 40-60μm and develop a few septa (Fig. 1, b). These hyphal branches are wider below and gradually taper above to a pointed tip. The lowest cell of each of these hyphae swells and becomes a globose to subglobose or elongate structure, the ascogonium (Fig. 1, c-h; Plate I: 1). The basal part of the setose structure becomes the stalk, while the terminal portion remains as a pointed free end of the ascogonium. Several ascogonia are produced within a stroma; they do not appear at the same time, but successively and in an irregular manner. Hyphal branches may arise from the stalk of each ascogonium and the basal part of its setose portion (Fig. 1, g). No antheridium was observed.

As the ascogonia develop within the stromatic tissue, the hyphal branches at the peripheral region of the stroma develop intercalary or terminal swellings (Fig. 1, i; Fig. 2, a-c, f-i). Each of these swollen hyphal portions fully expands and, in due course, develops a thick wall layer enclosing a central lumen; these

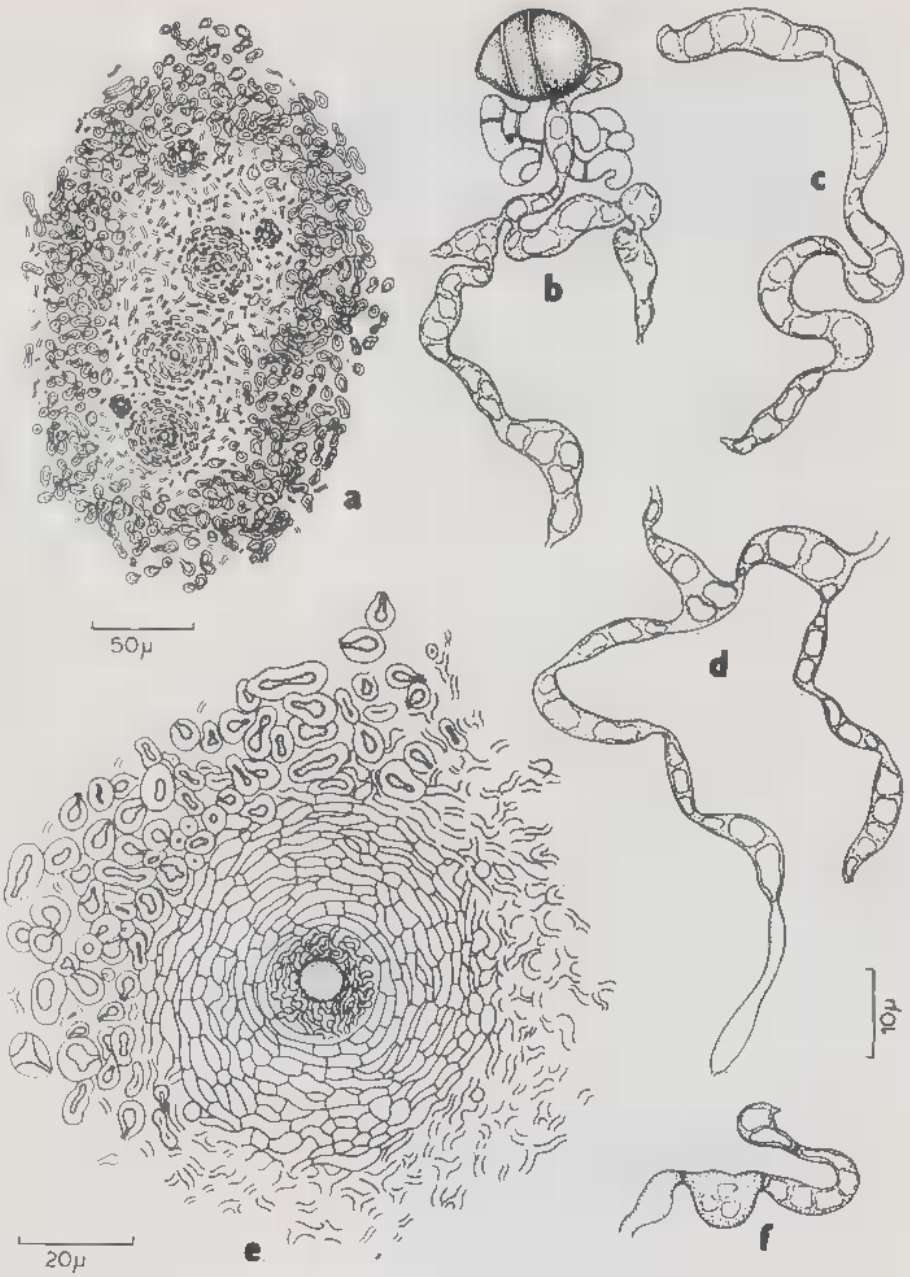


Fig. 3. — *Fennellia flavipes*. a: stroma containing young ascocarps (section) - note the conspicuous development of Hülle cells all around; b: ■ septate ascogonium, ascogenous hyphae and croziers; c, d: ascogenous hyphae; e: developing ascocarp showing ascogenous hyphae around ascogonium - note multilayered ascocarp wall (section); f: ■ portion of a septate ascogenous hypha - note the enlarged cell.

become the characteristic Hülle cells (Fig. 2, j-z). Sometimes, before or during the formation of these hyphal swellings, hyphal fusions appear to occur between adjacent hyphae so that a hyphal network is formed (Fig. 2, d & e). Swellings may then develop at regular or irregular intervals on these hyphae. Each swelling develops in a Hülle cell. The characteristically thick wall of the Hülle cell is apparently laid down centripetally, but the mechanism of wall formation is not fully understood. The shape of the Hülle cells varies from globose to elongate or sometimes irregular, and the Hülle cells form branched chains (Fig. 2, a-z'). Collectively, the Hülle cells constitute a covering for the stroma (Fig. 3, a).

The next stage in the development leads to the formation of the ascocarps within the stroma. As Hülle cells formation proceeds in the peripheral part of the stroma, the ascogonia produced within the stromatic tissue attain their characteristic shape and size. The hyphae surrounding each ascogonium now branch more densely and develop more septa. They also appear now more compactly arranged around each ascogonium than before and form a thick, several-layered outer covering around the ascogonium (Fig. 1, j-m; Plate I, 4). In the formation of this dense covering, the lateral branches produced from the stalk and the terminal portion of the ascogonium may also participate. There is continuous addition of Hülle cells all round. The size of the developing ascocarps within the stroma increases, followed by increase in thickness of the wall layers of the ascocarps. A section of the stroma at this stage shows several scattered young ascocarps in various stages of development enveloped by the fully developed Hülle cells (Fig. 3, a: Plate I, 3).

During the further development of the ascocarps, each ascogonium is cut off into two or three cells by the formation of one or two septa (Fig. 3, b). From any point on each of these cells, groups of hook-like structures (the croziers) and also a few narrow, thin and elongated hyphae (the ascogenous hyphae) develop (Fig. 3, b). Later, the ascogenous hyphae assume a curious aspect, being wider here and narrower there, become profusely branched, and grow around the ascogonium (Fig. 3, c-f). As the growth of the ascogenous hyphae around the ascogonium proceeds, the croziers produced directly from the ascogonium may also proliferate further to produce secondary or tertiary croziers, etc. (Fig. 5, a). Now the inner wall layers of the ascocarps slowly disintegrate resulting in the formation of a cavity around the ascogonium. Within this cavity the ascogenous hyphae grow further and crozier formation continues (Fig. 4, a & b; Plate I, 5 & 6).

Finally a stage is reached when there is no further extension or branching of the ascogenous hyphal system. At this stage, the ascogonium which lies embedded within the weft of the ascogenous hyphae slowly disintegrates. Certain portions of the ascogenous hyphae now become swollen and are cut off into individual cells by the formation of septa (Fig. 3, f). From each of these cells, another group of croziers develops, the morphology of which is the same as that of those produced directly from the ascogonium (Fig. 5, b).

The next stage in the development of the ascocarp leads to the formation of asci. Asci are produced either from croziers formed directly from the asco-

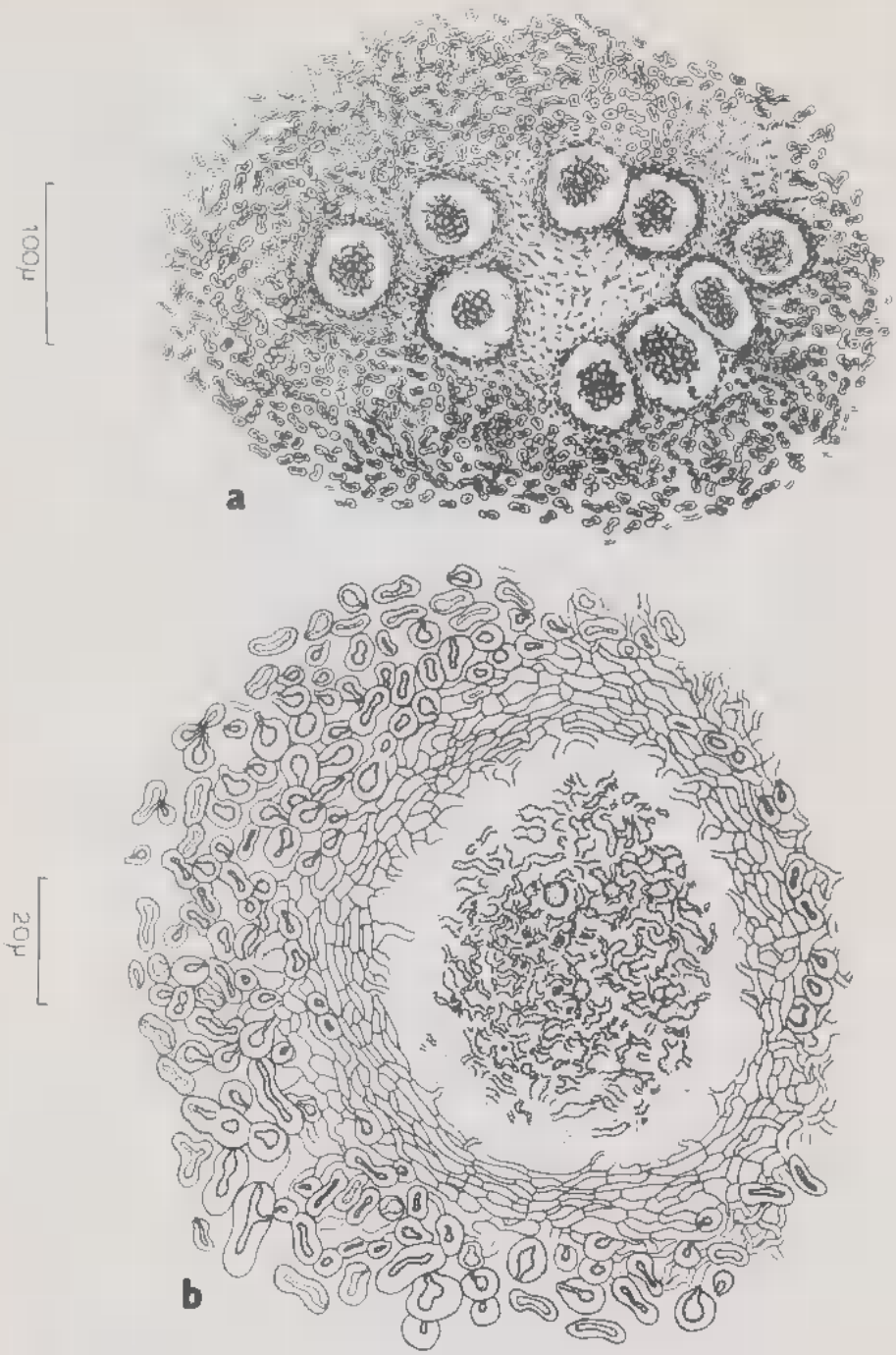


Fig. 4. – *Fennellia flavipes*. a: later stage in the development of ascocarps within a stroma (section); b: ascocarp showing ascogenous hyphae in the central part.

gonium or from croziers formed from the ascogenous hyphae; from both these groups of croziers, asci appear to be produced more or less at the same time. By septation, each crozier is divided into three cells: the basal, the penultimate and the tip cell (Fig. 5, c). The penultimate cell of the crozier enlarges and becomes the young ascus (Fig. 5, d-f). No case of fusion between the basal and the tip cells of the crozier was observed. The young ascus usually has a large vacuole in or about the centre, so that the protoplasm is confined to the periphery of the ascus (i. e., close to the ascus wall). Later, 4 or 8 ascospores become differentiated within each ascus.

Since a group of asci develops from each group of croziers (Fig. 5, i), the asci developing from different groups of croziers naturally lie scattered irregularly throughout the central cavity of the ascocarp (Fig. 5, h). The tip cell of each crozier usually remains attached to the related ascus even after its maturation, giving the appearance of a stalk (Fig. 5, j & k). The asci are thick-walled when young, thin-walled at maturity. Finally, the asci deliquesces so that the ascospores get released into the central cavity of the ascocarp. No ostiole was observed for the ascocarp.

As these developments occur, the stroma (with its Hülle cells covering) attains its final size and characteristic shape. Concurrently, the individual ascocarps within the stroma enlarge further and, at maturity, get pressed against each other so that their wall layers get compressed and the ascocarps coalesce; as a result, it might appear as if two neighbouring ascocarps have common wall layers. A section of the mature stroma at this stage may show several mature ascocarps united and surrounded by Hülle cells (Fig. 6, a; Plate 1, 7); and each ascocarp having a peridium of a few layers of thin-walled elongated narrow cells (Fig. 6, b).

Ascospore germination was not observed in spite of repeated study.

DEVELOPMENT OF THE *ASPERGILLUS* STATE

The development of the *Aspergillus* state starts with the production of phialophores. The phialophores arise as small protuberances from specialized hyphal cells, the foot cells (Fig. 7, a). The phialophore grows to varying lengths before forming a vesicle at its tip. As growth ceases, the apex of the phialophore swells to form a globose to subglobose vesicle (Fig. 7, c). The wall of the phialophore and the vesicle increases in thickness. Occasionally, the phialophore may develop a few septa. The mature phialophores are simple, erect or slightly bent and thick-walled.

When the vesicle has attained its final size and shape, minute protuberances arise from its surface (Fig. 7, d) possibly synchronously. Each protuberance swells and elongates and is cut off from the vesicle by a septum (Fig. 7, e). The protuberances elongate further (Fig. 7, f) and a second septum now cuts off each of them into an apical and a basal cell (Fig. 7, g). The basal cell corresponds to the metula; the apical cell is the phialide. A second phialide is now produced as a lateral extension from the metula just below the septum separa-

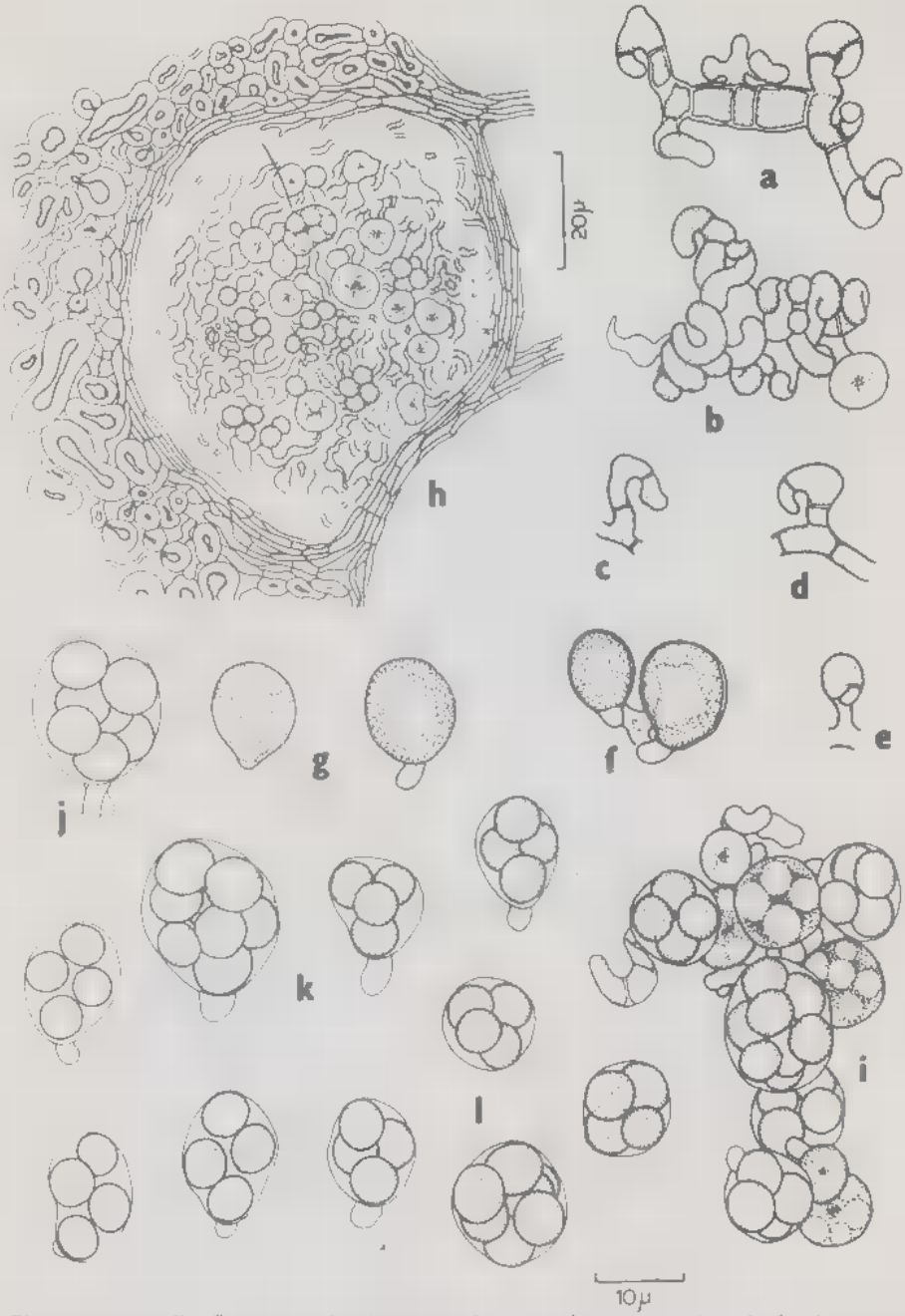


Fig. 5. *Fennellia flavipes*. a: development of croziers from ascogonium; b: development of a group of croziers from an ascogenous hypha; c-f: ascus development by crozier formation - note the central large vacuole in each young ascus in fig. f; g: young asci with the persistent tip cell of the crozier; h: ascocarp with developing asci (section); i: group of asci; j-l: mature asci.

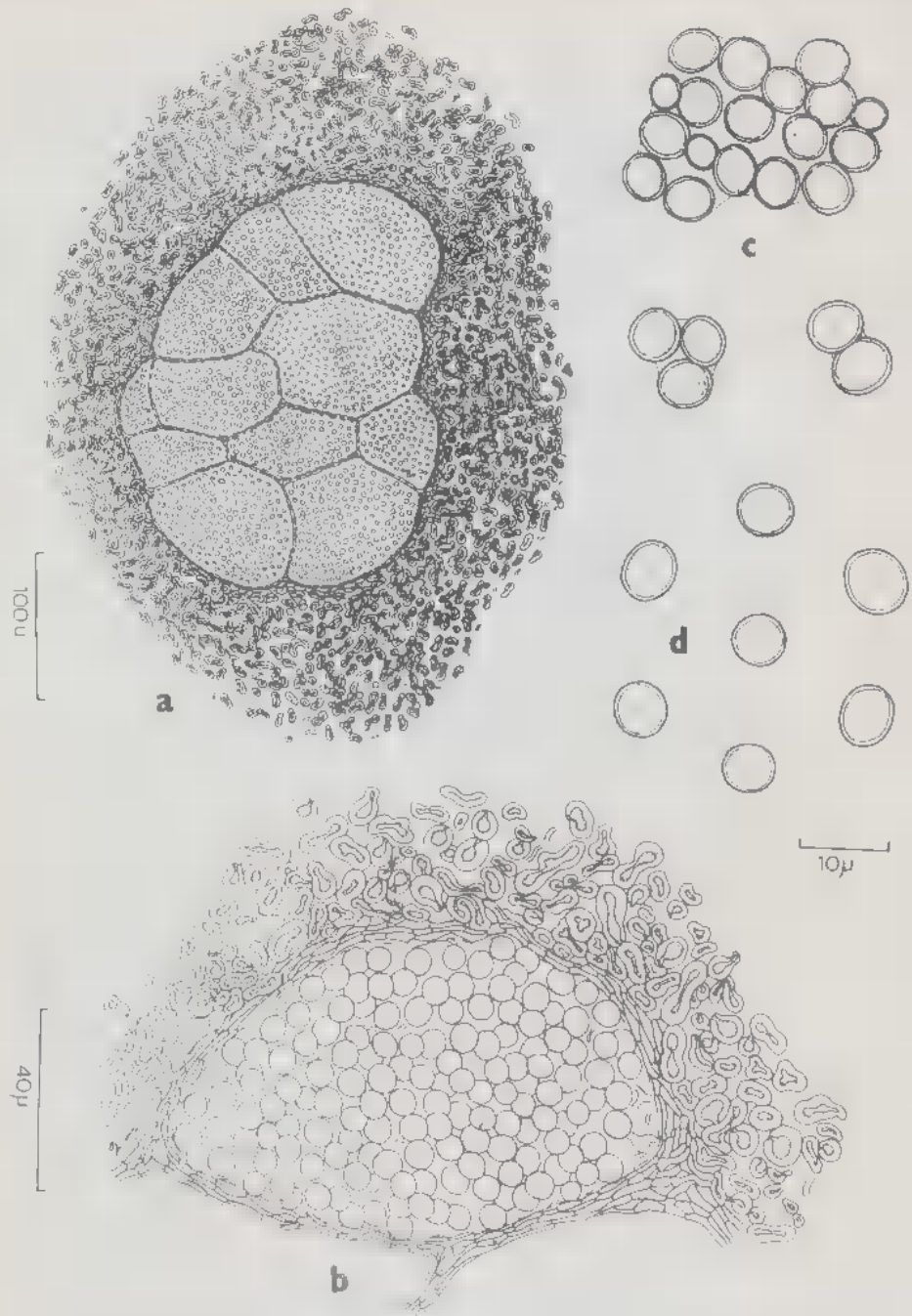


Fig. 6. - *Fennellia flavipes*. a: mature stroma with a group of several ascocarps (section); b: single ascocarp enlarged to show ascospores, multilayered peridium and Hülle cells (section); c: ascospores tending to stick together in clumps; d: mature ascospores.

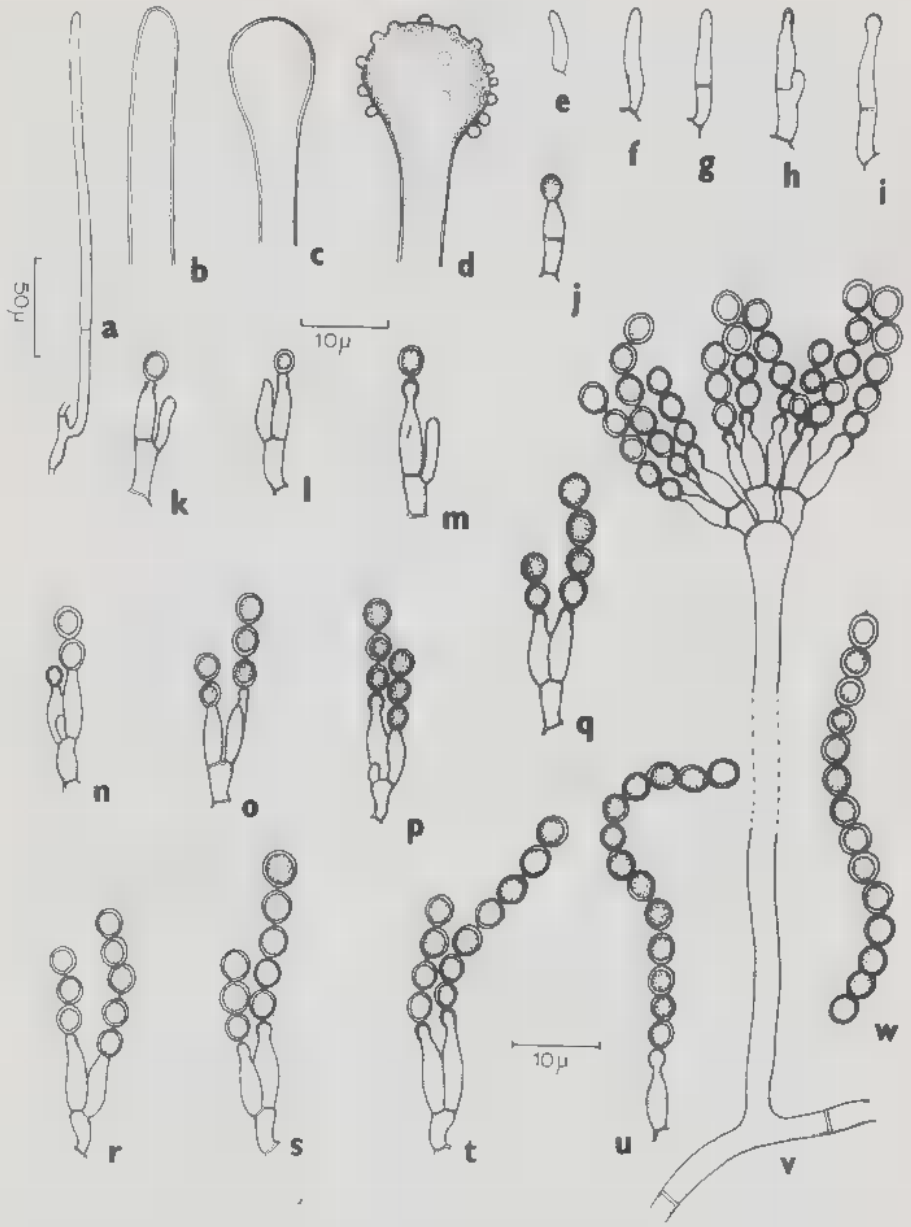
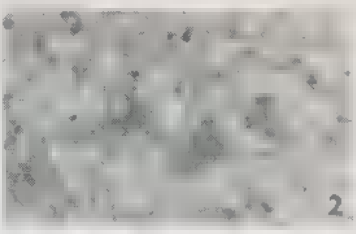
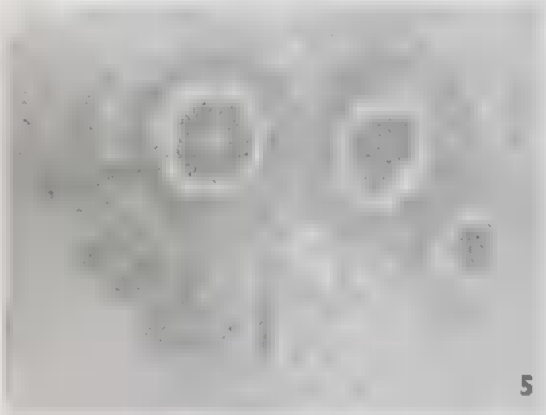


Fig. 7. *Fennellia flavipes*. a-h: development of phialophore, terminal vesicle, metulae and phialides; i-k: development of the first conidium - note rupture of phialide wall in fig. i and development of a second phialide on metula in fig. k; l-u: development of basipetal chain of conidia - note distinct isthmus between adjacent conidia; v: a mature phialophore with ▪ conidial head; w: a chain of conidia.





rating it from the first phialide; this phialide is eventually cut off by a septum (Fig. 7, h & k). This process is repeated so that several phialides are borne on each metula.

The development of the first phialide from each of the metulae on a vesicle appears to be synchronous; similarly, the development of the second phialide from each of the metulae, in most cases, also appears to be synchronous.

The conidia develop as follows: the initial of the first conidium appears as a small protuberance at the apex of each phialide (Fig. 7, i). The protuberance increases in size and becomes the initials of the first conidium. During further development the wall of the protuberance which is continuous with that of the phialide wall breaks at a presumably weak point located between the swollen tip and the body of the phialide (Fig. 7, j & k). As the first conidium is differentiated, a second conidium initial appears below the first conidium (Fig. 7, l), a third one below the second, and so on, so that a basipetal chain of conidia is produced (Fig. 7, m-u). Each conidium in the chain is separated from adjacent conidia by an isthmus. The isthmi are small and sometimes inconspicuous. The first formed conidium in a chain, usually has the part of the wall of the protuberance within which it has first differentiated as an inconspicuous cap enveloping it (Fig. 7, j). The region of the break of the wall of the protuberance corresponds in diameter to the width of the open end of the phialide itself, as this is the region where it got broken off from the phialide apex. No collarette is observed at the open and of the phialide.

TAXONOMY

The genus *Fennellia* Wiley and Simmons (WILEY & SIMMONS, 1973) is based on *F. flavipes* Wiley and Simmons. The development of the teliomorph and anamorph of this fungus was studied from type material. The results presented here are the first detailed account of the developmental morphology of this species.

The ascocarps develop within a mass of loosely interwoven narrow hyphae. This mass of loosely interwoven hyphae is interpreted here as an ascostroma which is prosenchymatous. The ascocarps are found to occur in groups enveloped by numerous Hülle cells which collectively constitute a hard, thick outer covering. Each ascocarp has its own pseudoparenchymatous peridium of 2-4 layers of cells. In its structure, therefore, this fungus is unique among the species

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Plate I. -- *Fennellia flavipes*. 1: stages in the development of ascogonia, x 1400. 2: sectional view of 2 mature ascogonia in a stroma, x 1050. 3: stroma containing young ascocarps (section), x 110. 4: stages in the development of ascocarps in a stroma (section). Note ascogonium in the centre in each ascocarp, x 1420. 5: a portion of a stroma (enlarged) showing developing ascocarps, x 960. 6: a stroma containing young ascocarps (section). Note the conspicuous development of Hülle cells all around, x 180. 7: mature stroma with a group of several ascocarps (section) with free ascospores within, x 330. 8: a typical mature phialophore showing the conidial head, x 440.

we have discussed so far. The initiation of the ascocarp is by the development of several scattered ascogonia within the distinct mass of hyphae (i. e. the stroma) already referred to. The ascogonia are peculiar, globose to subglobose or sometimes elongate, each with a short stalk and a straight, septate trichogyne-like extension (Fig. 1, c-f). As far as we know, such ascogonia have not been reported for any of the teliomorph of *Aspergillus* and so are interesting. Each ascocarp is the product of development of one ascogonium. The asci are produced from croziers on ascogenous hyphae which have a characteristic morphology (Fig. 3, c & d). Croziers have also been seen on what appear to be very short ascogenous hyphae arising from the ascogonium. Croziers may also arise directly from the ascogonium.

The developmental morphology of the anamorph of *Fennellia flavipes* has been studied in detail here and it is a good *Aspergillus*.

The conspicuous presence of Hülle cells in *Fennellia flavipes* at once recalls the genus *Emericella* Berk. and Br. in which also the ascocarps are enmeshed by Hülle cells. Unfortunately, *Emericella* was not investigated by the author. No detail account of the developmental morphology of the type species, *Emericella variecolor* Berk. and Br., is available for comparison. Therefore, we have to rely entirely on informations available in EIDAM's (EIDAM, 1883) paper on *E. nidulans* Eidam. From this observations, it would appear that the ascocarp in *E. nidulans* has a pseudoparenchymatous peridium and the asci are globose to subglobose, avanescent and scattered within the ascocarp. In these features, *Fennellia flavipes* resembles *Emericella nidulans*. However, from EIDAM's description, the ascogonia in *Emericella nidulans* are not globose. The difficulty is : for a proper comparison with *Emericella*, we must have complete informations on the developmental morphology of its type species, *E. variecolor*. Unfortunately, as already indicated, no such information is available. If the ascogonium in *E. variecolor* proves to be similar to that in *Fennellia flavipes* and, additionally, the ascocarps are found to develop in groups or singly in a stroma becoming finally collectively or singly enclosed in an envelope of Hülle cells, the basic similarity in developmental morphology between *Fennellia* and *Emericella* would be complete. At present, however, we need further informations on important features of the developmental morphology of *Emericella variecolor* to find support for this possible conclusion (though the ascospores of *E. variecolor* are quite distinct from those of *F. flavipes* in that they are crested in the former, but only grooved in the latter).

We are grateful to Dr. E.G. Simmons for the type culture of *Fennellia flavipes* on which the present study is based.

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